Clin. exp. Immunol. (1990) **81**, 319–324

In vitro production of tumour necrosis factor and prostaglandin E_2 by peripheral blood mononuclear cells from tuberculosis patients

J. CADRANEL, C. PHILIPPE, J. PEREZ, B. MILLERON, G. AKOUN, R. ARDAILLOU & L. BAUD
INSERM U.64 and Service de Pneumo-Phtisiologie et Réanimation Respiratoire, Hôpital Tenon, Paris, France

(Accepted for publication 22 February 1990)

SUMMARY

We investigated the production of tumour necrosis factor-alpha (TNF-α) and prostaglandin E_2 (PGE_2) by peripheral blood mononuclear cells (PBMC) from tuberculosis patients and healthy controls. PBMC from tuberculosis patients generated constitutively more TNF-α than did control PBMC. This production was significantly higher for patients with high-grade fever and cachexia. The increase of TNF-α production by PBMC from tuberculosis patients was associated with a comparatively weaker elevation of PGE_2 synthesis which did not parallel fever or weight loss. *In vitro* treatment of control PBMC with the tuberculin purified protein derivative (PPD) promoted an increased TNF-α production which was similar to that of untreated PBMC from tuberculosis patients. Thus, the increased TNF-α production in tuberculosis could be explained by the *in vivo* exposure of PBMC to mycobacterial antigens. In contrast, the concentration of PGE_2 was weaker in the medium of untreated PBMC from tuberculosis patients than in the medium of PPD-treated control PBMC, suggesting that PGE_2 synthesis by PBMC was limited in tuberculosis by unidentified factors.

Keywords tuberculosis tumour necrosis factor prostaglandin blood mononuclear cells

INTRODUCTION

Several recent studies suggest that local synthesis and release of tumour necrosis factor (TNF) may be involved in the granuloma formation. Macrophages isolated from pulmonary granuloma induced by Sephadex beads release TNF (Chensue *et al.*, 1989). Similarly, TNF is synthesized by activated macrophages accumulating in the well-developed liver granulomas during BCG infection (Kindler *et al.*, 1989). In this experimental model, injection of bacterial lipopolysaccharide (LPS) determines an increase of TNF concentration in the serum (Green *et al.*, 1976) which is proportional to the expression of TNF mRNA in granulomas (Kindler *et al.*, 1989). This suggests that macrophages involved in granuloma formation are likely to be the major source of serum TNF. However, in tuberculosis, which is also characterized by granulomatous inflammation (Williams & Williams, 1983), TNF could be released from the local microenvironment of granulomas and probably also from circulating monocytes (Strieter *et al.*, 1989) and/or lymphocytes (Christmas, Meager & Moore, 1987; Cuturi *et al.*, 1987; Steffen, Ottmann & Moore, 1988). Indeed, T lymphocytes from tuberculosis patients which are responsive to the tuberculin purified protein derivative (PPD), circulate between lymphoid organs and the peripheral blood and produce lymphokines stimulating several monocyte functions (Ellner, Spagnuollo & Schachter, 1981; Edwards & Kirkpatrick, 1986); in addition, mycobacterium tuberculosis *per se* or purified polysaccharides from *Mycobacterium tuberculosis* can induce *in vitro* TNF release from peripheral blood mononuclear cells (PBMC) (Rook *et al.*, 1987; Valone *et al.*, 1988; Moreno *et al.*, 1989). However, to our knowledge, the capacity of PBMC from tuberculosis patients to produce TNF has not yet been examined.

We therefore set to compare the release of TNF-α by PBMC from tuberculosis patients and from a control group of healthy blood donors. Since polysaccharides from *M. tuberculosis* increase mononuclear cell prostaglandin E_2 (PGE_2) production (Kleinhenz *et al.*, 1981), which in turn reduces the TNF-α production by these cells through a rise of cAMP (Kunkel *et al.*, 1988; Spengler *et al.*, 1989a, 1989b; Hart *et al.*, 1989a, 1989b; Heidenreich *et al.*, 1989), we sought to determine the release of PGE_2 by PBMC under the same conditions.

MATERIALS AND METHODS

Patient population

Blood mononuclear cells were recovered from 16 patients (aged 34 ± 11 years) with pulmonary tuberculosis and 14 healthy subjects (aged 35 ± 8 years). The diagnosis of pulmonary tuberculosis was established on the basis of cultures of sputum or gastric aspirates positive for *M. tuberculosis* ($n = 10$) and/or

Correspondence: Dr Laurent Baud, INSERM U.64, Hôpital Tenon, 4, rue de la Chine, 75970 Paris Cedex, France.

the presence of epithelial granuloma with caseous necrosis on histological study of biopsy specimens ($n=7$). Tuberculin skin test was strongly positive in all patients. Five patients had a high grade fever (>38·5°C) and a weight loss of >6 kg; 11 patients had a low grade fever (<38°C) and a weight loss of <5 kg. Results of chest radiographs were as follows: parenchymal infiltrates with or without cavitation ($n=10$); hilar adenopathy without parenchymal infiltrates ($n=6$). Patients were studied prior to receiving any anti-tuberculous therapy.

Cell cultures

Blood samples from each subject were diluted 1/1 (vol/vol) in calcium-free HBSS supplemented with 0·08% EDTA, and mononuclear cells were isolated by density centrifugation on Lymphopaque (Nyegaard, Oslo, Norway). The mononuclear cells were resuspended in the same medium diluted 1/9 (vol/vol) in calcium-free HBSS and centrifuged (100 *g* for 10 min) twice to remove the platelets. Thereafter, PBMC were resuspended in culture medium consisting of RPMI 1640 (Flow Laboratories, Irvine, UK) buffered with 20 mM HEPES to pH 7·4, and supplemented with 1% decomplemented fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine, counted after staining with acridine orange, and adjusted to a concentration of 10^6 cells/ml. One-hundred-microlitre volumes of this suspension were added to 96-well microplates (Nunc, Roskilde, Denmark). The total volume of each well was adjusted to 200 μl with complete medium and different stimuli used in the following final concentrations: lipopolysaccharide (LPS) from *Escherichia Coli* 026B6 (Sigma Chemical Co., St Louis, MO), 1 μg/ml; PPD (Institut Pasteur, Paris, France), 5 μg/ml; phytohaemagglutinin (PHA) (Wellcome, Beckenham, UK), 1 μg/ml. Where indicated, PBMC were simultaneously treated with 10 μM indomethacin (Sigma). The periods of incubation and the concentrations chosen were based on the time-course of TNF-α release observed in preliminary experiments under each condition; a maximal TNF production occurred at 18 h in the absence of challenge or in the presence of LPS, and at 72 h in the presence of PPD or PHA. After incubation of the cells, cell-free supernatants were frozen at −70°C until analyzed for TNF-α and PGE_2.

Tumour necrosis factor assay

TNF-α released from PBMC was assessed by both TNF-α immunoradiometric assay and TNF-α bioassay. For the immunoradiometric assay, 25–100 μl of cell culture medium were incubated overnight with ^{125}I-labelled anti-TNF-α monoclonal antibody in tubes coated with a monoclonal antibody directed against different epitopes of TNF-α (TNF-α- IRMA; Ire-Medgenix, Fleurus, Belgium). After washing, the remaining radioactivity bound to the tube was counted in a gamma counter. In additional experiments, the activity of TNF-α was also determined in the cell culture medium using a L-929 fibroblast lytic assay as previously described (Baud *et al.*, 1989). For neutralization studies, test samples were mixed with anti-human TNF-α (Genzyme, Boston, MA) used in excess of the concentration that should provide maximum inhibition. Samples were assayed in triplicate.

PGE_2 assay

Radioimmunoassay of PGE_2 was carried out in the culture medium of PBMC, at four increasing dilutions by using ^{3}H-PGE_2 from the Radiochemical Centre (Amersham, UK) and anti-PGE_2 antibody from Institut Pasteur. This antibody cross-reacts only slightly with other PGs and could be considered as specific (Sraer *et al.*, 1979).

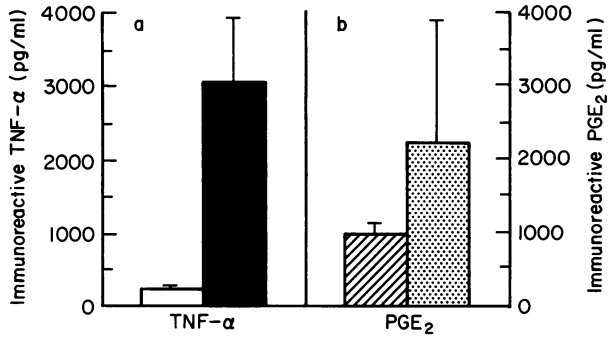

Fig. 1. (a) Production of TNF-α by untreated PBMC obtained from tuberculosis patients (■) and healthy donors (□); (b) production of PGE_2 by untreated PBMC obtained from tuberculosis patients (▨) and healthy donors (■). Concentrations in supernatants from PBMC cultured for 18 h, expressed as mean + s.e.m.

Statistical analysis

The data were analysed using Student's *t*-test for paired or unpaired values. Regression analysis was performed to estimate the relationship between two parameters.

RESULTS

Spontaneous release of TNF-α and PGE_2 by mononuclear cells from the tuberculosis and control groups

The proportion of monocytes in the PBMC population was similar in the tuberculosis group (30·3 ± 9·2%) and in the control group (39·1 ± 5·8%). Despite this, the TNF-α concentrations determined by IRMA in the cell-free supernatants of PBMC were much higher (14-fold) in the tuberculosis group (Fig. 1). This difference was statistically significant ($P<0·01$). When the supernatants were tested in the TNF-α bioassay, a highly significant correlation ($r=0·85$; $P<0·001$) was found between the results obtained with this assay and those of the TNF-α-IRMA, at least for the TNF-α concentrations higher than the threshold of sensitivity of the TNF-α bioassay. We have also determined which concentrations of PGE_2 were reached in the cell-free supernatants of PBMC, and to what extent PGE_2 affected TNF-α release from PBMC (Renz *et al.*, 1988; Spengler *et al.*, 1989a, 1989b). A slightly higher (two-fold) concentration of PGE_2 was observed in the medium of PBMC of the tuberculosis group than in that of the control group ($P=0·05$) (Fig. 1). In the tuberculosis group there was a positive correlation between the levels of TNF-α and PGE_2 at least for the PGE_2 concentrations below 4000 pg/ml ($r=0·83$; $P<0·001$) (Fig. 2). For the two highest values, TNF-α production was almost completely suppressed. In the control group, no correlation was observed. To determine the potential regulatory effect of PGE_2 on TNF-α production, supplementary experiments were performed using indomethacin, a cyclo-oxygenase inhibitor. Figure 3 shows that indomethacin induced an increased release of TNF-α by PBMC of the control group ($P<0·02$) but did not affect significantly that by PBMC of the tuberculosis group.

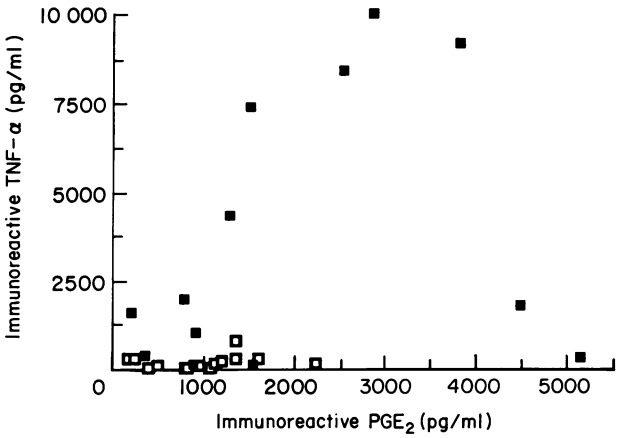

Fig. 2. Relationship between TNF-α and PGE_2 concentrations in the supernatants of PBMC obtained from tuberculosis patients (■) and healthy donors (□) and cultured for 18 h.

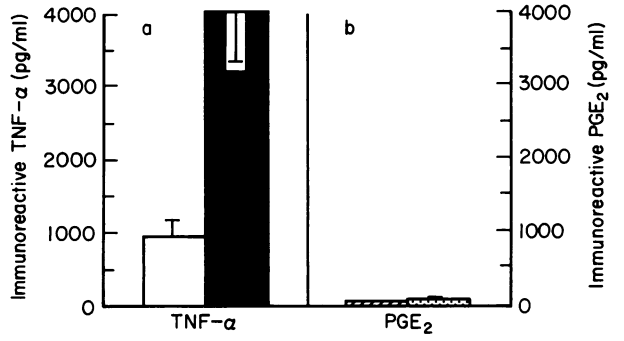

Fig. 3. (a) Production of TNF-α by indomethacin-treated PBMC obtained from tuberculosis patients (■) and healthy donors (□); (b) Production of PGE_2 by indomethacin-treated PBMC obtained from tuberculosis patients (▨) and normal donors (■). Concentrations in supernatants from PBMC cultured for 18 h, expressed as mean ± s.e.m.

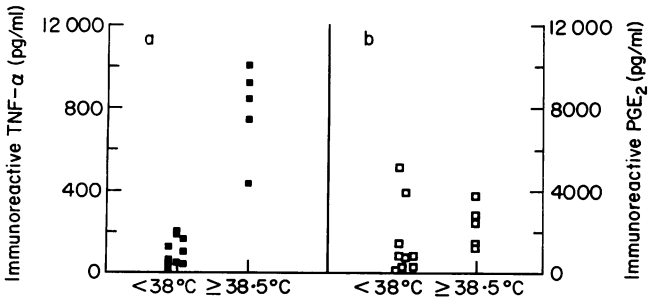

Fig. 4. Relationship between the incidence of fever and the production of TNF-α (■) or PGE_2 (□) by untreated PBMC obtained from tuberculosis patients. Concentrations in supernatants from PBMC cultured for 18 h.

In the tuberculosis group, high levels of TNF-α in the cell-free supernatants of PBMC were significantly related to the severity of fever (Fig. 4) ($P < 0{\cdot}001$) and of weight loss (data not shown), these two parameters being strongly associated. However, there was no correlation between TNF-α concentrations and blood cell counts, positivity of cultures for *M. tuberculosis*, presence of cavitation on chest radiographs, or extension of the disease. Similarly, there was no correlation between PGE_2 concentrations and either of these parameters.

Release of TNF-α and PGE_2 by mononuclear cells from the control group stimulated in vitro *with PPD*

It has not been established whether the altered capacity of PBMC from tuberculosis patients to release TNF-α and PGE_2 is caused by *in vivo* exposure of these cells to mycobacterial antigens. To answer this question, we sought to determine the *in vitro* effect of PPD on TNF-α and PGE_2 synthesis by PBMC from the control group. PBMC were exposed for 1–3 days to PPD (5 μg/ml) and for comparison to LPS (1 μg/ml) or PHA (1 μg/ml). PPD as well as the two other agents promoted an increased release of TNF-α and PGE_2 (Fig. 5). It was of particular note that PPD treatment enhanced more markedly PGE_2 release (30-fold) than TNF-α production (four-fold). Thus the concentrations of TNF were similar in the supernatants of untreated PBMC from tuberculosis patients (Fig. 1) and in those of control PBMC exposed *in vitro* to PPD (Fig. 5). In contrast, PGE_2 concentrations were much lower in the supernatants of untreated PBMC from tuberculosis patients (Fig. 1) than in those of control PBMC exposed *in vitro* to PPD ($P < 0{\cdot}001$) (Fig. 5). This finding suggested that a part of the immune PBMC dysfunction in tuberculosis was related to a decreased capacity to release PGE_2 in response to mycobacterial antigens.

Release of TNF-α and PGE_2 by mononuclear cells from the tuberculosis group stimulated in vitro *with PHA and PPD*

To verify the decreased capacity of PBMC from tuberculosis patients to release PGE_2, we examined whether exposure of these cells to an *in vitro* challenge was associated with a low production of PGE_2. As shown in Fig. 6, the increase of PGE_2 concentration observed in the supernatant of PBMC upon exposure to PHA was weaker for the tuberculosis group than for the control group (5898 ± 1579 pg/ml and 13 280 ± 3450 pg/ml, respectively; $P < 0{\cdot}05$). In contrast, the increase of TNF-α concentration was higher for the tuberculosis group than for the control group (14 879 ± 2495 pg/ml and 3113 ± 582 pg/ml, respectively; $P < 0{\cdot}001$). Similar results were obtained upon PBMC stimulation with PPD (data not shown). This indicated that PBMC from tuberculosis patients exhibited a constitutive failure of PGE_2 production when exposed to different *in vitro* challenges.

DISCUSSION

Our results demonstrate that PBMC from tuberculosis patients produce significantly more TNF-α than do cells from control subjects. This conclusion is based on analysis of *in vitro* TNF-α secretion using both a sensitive highly specific TNF-α-IRMA and a TNF-α bioassay. Only functionally active TNF-α was detected. Thus its synthesis by PBMC from tuberculosis patients may play a pathophysiologic role *in vivo*.

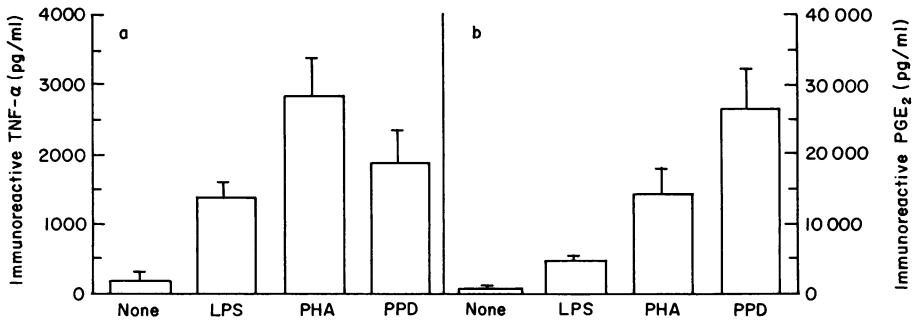

Fig. 5. Production of TNF-α (a) and PGE_2 (b) by PBMC obtained from healthy donors, in response to LPS, PHA, or PPD. Concentrations in supernatants from PBMC cultured for 18 h (LPS) or 72 h (PHA, PPD), expressed as mean + s.e.m.

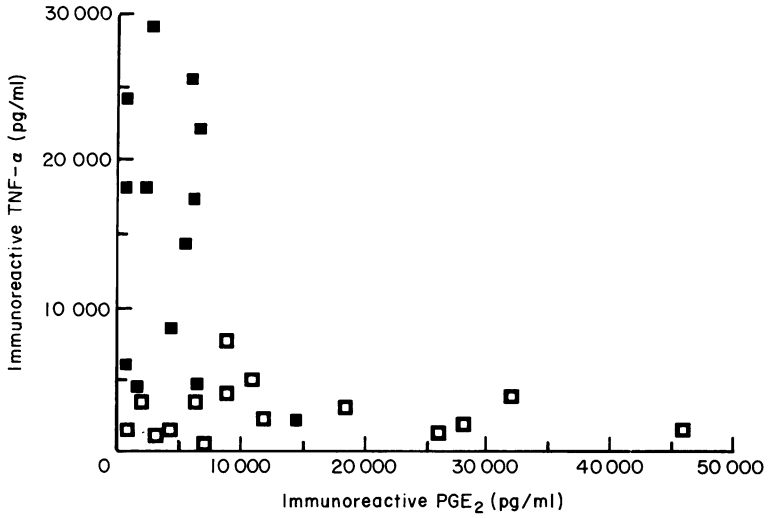

Fig. 6. Relationship between TNF-α and PGE_2 concentrations in the supernatants of PBMC obtained from tuberculosis patients (■) and healthy donors (□) exposed for 72 h to PHA.

In tuberculosis, an increased monocytopoiesis occurs resulting in the release of monocytes from the bone marrow (Edwards & Kirkpatrick, 1986). Nevertheless, in our study the proportion of monocytes in the PBMC fraction obtained by the centrifugation on Lymphopaque was not increased but rather decreased weakly in the tuberculosis group. Therefore, the augmented TNF-α secretion cannot be due to an increased proportion of monocytes in the peripheral blood but rather to the augmented TNF-α secretion per mononuclear cell, monocyte and/or lymphocyte (Cuturi *et al.*, 1987).

The observation that PBMC constitutively produce high amounts of TNF-α is not specific of pulmonary tuberculosis since similar results have been reported previously in the course of other infectious diseases characterized by fever and weight loss, such as HIV-1 (Wright *et al.*, 1988; Roux-Lombard *et al.*, 1989), or *Yersinia* infections (Repo *et al.*, 1988). Consistent with this concept, the present results demonstrate that TNF-α production by PBMC was significantly related to the severity of fever and weight loss (Fig. 4). Nevertheless, the underlying mechanisms leading to the induction of fever in tuberculosis may also involve the production by PBMC of other cytokines such as interleukin-1 (IL-1), as previously reported by Chensue *et al.* (1986). In contrast, in pulmonary sarcoidosis, a disease also characterized by granuloma formation, the ability of PBMC to release TNF is not altered (Spatafora *et al.*, 1989), suggesting that an increased capacity of PBMC to synthesize TNF does not characterize all granulomatous diseases.

Although PBMC of all the tuberculosis patients studied showed a detectable TNF-α synthesis, the amount of TNF-α secreted varied considerably among patients. The production of TNF-α was significantly correlated with that of PGE_2 (Fig. 2). The reason may be that the *in vitro* synthesis of both products depends on the extent to what T lymphocytes were primed and monocytes were pre-stimulated *in vivo*. Surprisingly, the particularly high concentration of PGE_2 observed in PBMC culture supernatants from two patients was associated with a low level of TNF-α. This finding is in line with the concept that, at high concentrations, PGE_2 down-modulates TNF-α mRNA expression and hence TNF-α synthesis through a rise of its second messenger, cAMP (Renz *et al.*, 1988; Spengler *et al.*, 1989a, 1989b). In this context, the inability of indomethacin to increase TNF-α synthesis by PBMC from tuberculosis patients could be explained by two mechanisms. First, the constitutive PGE_2 production by PBMC from the majority of tuberculosis patients was too low to affect TNF-α synthesis (Renz *et al.*, 1988). Second, TNF-α mRNA expression and TNF-α synthesis probably started to occur *in vivo*; the subsequent *in vitro* treatment by indomethacin was too late to be effective since the ability of

PGE_2 to regulate the production of TNF-α does not exceed 3 h post-challenge (Spengler *et al.*, 1989a, 1989b).

Whether this PBMC activation was caused by their *in vivo* exposure to *M. tuberculosis* antigens is speculative. The studies reported here at least confirm the capacity of PPD, as other *M. tuberculosis* antigens, to increase the mononuclear cell production of TNF-α (Rook *et al.*, 1987; Valone *et al.*, 1988; Moreno *et al.*, 1989). Supplementary studies are needed, however, to determine whether mediator(s) involved in the immune response to tuberculosis antigens may participate in this PBMC activation.

Compared with the production of TNF-α, the synthesis of PGE_2 by PBMC from tuberculosis patients was weakly enhanced. This is surprising because the *in vitro* treatment of control PBMC by PPD was associated with a higher increase of PGE_2 than of TNF-α concentrations (Fig. 5). This observation indicates that, in tuberculosis, the capacity of PBMC to convert arachidonic acid into PGE_2 is limited by still unidentified factor(s). Among these factors, glucocorticoids and interleukin-4 both of which suppress PGE_2 production by human monocytes (Hart *et al.*, 1989a) could be adequate candidates. However, since they simultaneously reduce TNF-α synthesis (Waage & Bakke, 1988; Hart *et al.*, 1989a), their role in this context is unlikely. Whatever its mechanisms, the limitation of PGE_2 synthesis by PBMC may result *in vivo* in an enhanced cellular immune response through several ways. PGE_2 is indeed a potent inhibitor of several lymphocyte functions (mitogenesis, antibody and lymphokine production) (Goodwin & Webb, 1980; Stenson & Parker, 1980), which are involved in the immune response to tuberculosis infection (Edwards & Kirkpatrick, 1986).

Our conclusions are that the production of TNF-α by PBMC is increased in patients with pulmonary tuberculosis, particularly in those with a high degree of fever and weight loss; and that PBMC from tuberculosis patients exhibit high background PGE_2 and impaired PGE_2 response to stimuli *in vitro*, suggesting that PGE_2-mediated immunoregulation is abnormal in this disease.

ACKNOWLEDGMENTS

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, from the Faculté de Médecine Saint Antoine, and from the Programme National de Recherches sur le SIDA et les rétrovirus humains. We thank Mrs Miranda, Mrs Morin, and Mrs Knobloch for secretarial assistance.

REFERENCES

BAUD, L., OUDINET, J.P., BENS, M., NOE, L., PERALDI, M.N., RONDEAU, E., ETIENNE, J. & ARDAILLOU, R. (1989) Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. *Kidney Int.* **35,** 1111.

CHENSUE, S.W., DAVEY, M.P., REMICK, D.G. & KUNKEL, S.L. (1986) Release of interleukin-1 by peripheral blood mononuclear cells in patients with tuberculosis and active inflammation. *Infect. Immun.* **52,** 341.

CHENSUE, S.W., OTTERNESS, I.G., HIGASHI, G.I., FORSCH, C.S. & KUNKEL, S.L. (1989) Monokine production by hypersensitivity (*Schistosoma mansoni* egg) and foreign body (Sephadex bead)-type granuloma macrophages: evidence for sequential production of IL-1 and tumor necrosis factor. *J. Immunol.* **142,** 1281.

CHRISTMAS, S.E., MEAGER, A. & MOORE, M. (1987) Production of interferon and tumor necrosis factor by cloned human natural cytotoxic lymphocytes and T cells. *Clin. exp. Immunol.* **69,** 441.

CUTURI, M.C., MURPHY, M., COSTA-GIOMI, M.P., WEINMANN, R., PERUSSIA, B. & TRINCHIERI, G. (1987) Independent regulation of tumor necrosis factor and lymphotoxin production by human peripheral blood lymphocytes. *J. exp. Med.* **165,** 1581.

EDWARDS, D. & KIRKPATRICK, C.H. (1986) The immunology of mycobacterial diseases. *Am. Rev. respir. Dis.* **134,** 1062.

ELLNER, J.J., SPAGNUOLO, P.J. & SCHACHTER, B.Z. (1981) Augmentation of selective monocyte functions in tuberculosis. *J. infect. Dis.* **144,** 391.

GOODWIN, J.S. & WEBB, D.R. (1980) Regulation of the immune response by prostaglandins. *Clin. Immunol. Immunopathol.* **15,** 106.

GREEN, S., DOBRJANSKY, A., CARSWELL, E.A., KASSEL, R.L., OLD, L.J., FIORE, N. & SCHWARTZ, M.K. (1976) Partial purification of a serum factor that causes necrosis of tumors. *Proc. natl Acad. Sci. USA*, **73,** 381.

HART, P.H., VITTI, G.F., BURGESS, D.R., WHITTY, G.A., PICCOLI, D.S. & HAMILTON, J.A. (1989a) Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. *Proc. natl Acad. Sci. USA*, **86,** 3803.

HART, P.H., WHITTY, G.A., PICCOLI, D.S. & HAMILTON, J.A. (1989b) Control by IFN-γ and PGE_2 of TNFα and IL-1 production by human monocytes. *Immunology*, **66,** 376.

HEIDENREICH, S., GONG, J.H., SCHMIDT, A., NAIN, M. & GEMSA, D. (1989) Macrophage activation by granulocyte/macrophage colony-stimulating factor: priming for enhanced release of tumor necrosis factor α and prostaglandin E_2. *J. Immunol.* **143,** 1198.

KINDLER, V., SAPPINO, A.P., GRAU, G.E., PIGUET, P.F. & VASSALLI, P. (1989) The inducing role of tumor necrosis factor in the development of bactericidal granulomas during BCG infection. *Cell*, **56,** 731.

KLEINHENZ, M.E., ELLNER, J.J., SPAGNUOLO, P.J. & DANIEL, T.M. (1981) Suppression of lymphocyte responses by tuberculous plasma and mycobacterial arabinogalactan. *J. clin. Invest.* **68,** 153.

KUNKEL, S.L., SPENGLER, M., MAY, M.A., SPENGLER, R., LARRICK, J. & REMICK, D. (1988) Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. *J. biol. Chem.* **263,** 5380.

MORENO, C., TAVERNE, J., MEHLERT, A., BATE, C.A.W., BREALEY, R.J., MEAGER, A., ROOK, G.A.W. & PLAYFAIR J.H.L. (1989) Lipoarabinomannan from *Mycobacterium tuberculosis* induces the production of tumour necrosis factor from human and murine macrophages. *Clin. exp. Immunol.* **76,** 240.

RENZ, H., GONG, J.H., SCHMIDT, A., NAIN, M. & GEMSA, D. (1988) Release of tumor necrosis factor α from macrophages: enhancement and suppression are dose-dependently regulated by prostaglandin E_2 and cyclic nucleotides. *J. Immunol.* **141,** 2388.

REPO, H., JAATTELA, M., LEIRISALO-REPO, M. & HURME, M. (1988) Production of tumour necrosis factor and interleukin 1 by monocytes of patients with previous *Yersinia* arthritis. *Clin. exp. Immunol.* **72,** 410.

ROOK, G.A.W., TAVERNE, J., LEVETON, C. & STEELE, J. (1987) The role of gamma-interferon, vitamin D_3 metabolites and tumour necrosis factor in the pathogenesis of tuberculosis. *Immunology*, **62,** 229.

ROUX-LOMBARD, P., MODOUX, C., CRUCHAUD, A. & DAYER, J.M. (1989) Purified blood monocytes from HIV 1-infected patients produce high levels of TNFα and IL-1. *Clin. Immunol. Immunopathol.* **50,** 374.

SPATAFORA, M., MERENDINO, A., CHIAPPARA, G., GJOMARKAJ, M., MELIS, M., BELLIA, V. & BONSIGNORE, G. (1989) Lung compartmentalization of increased TNF releasing ability by mononuclear phagocytes in pulmonary sarcoidosis. *Chest* **96,** 542.

SPENGLER, R.N., SPENGLER, M.L., STRIETER, R.M., REMICK, D.G., LARRICK, J.W. & KUNKEL, S.L. (1989a) Modulation of tumor necrosis factor-α gene expression. Desensitization of prostaglandin E_2-induced suppression. *J. Immunol.* **142,** 4346.

SPENGLER, R.N., SPENGLER, M.L., LINCOLN, P., REMICK, D.G., STRIETER, R.M. & KUNKEL, S.L. (1989b) Dynamics of dibutyryl cyclic AMP- and prostaglandin E_2-mediated suppression of lipopolysaccharide-induced tumor necrosis factor alpha gene expression. *Infect. Immun.* **57,** 2837.

SRAER, J., FOIDART, J., CHANSEL, D., MAHIEU, P., KOUZNETZOVA, B. & ARDAILLOU, R. (1979) Prostaglandin synthesis by mesangial and epithelial glomerular cultured cells. *FEBS Lett.* **104,** 420.

STEFFEN, M., OTTMANN, O.G. & MOORE, M.A.S. (1988) Simultaneous production of tumor necrosis factor-α and lymphotoxin by normal T cells after induction with IL-2 and anti-T3. *J. Immunol.* **140,** 2621.

STENSON, W.F. & PARKER, C.W. (1980) Prostaglandins, macrophages and immunity. *J. Immunol.* **125,** 1.

STRIETER, R.M., REMICK, D.G., LYNCH, J.P., GENORD, M., RAIFORD, C., SPENGLER, R. & KUNKEL, S.L. (1989) Differential regulation of tumor necrosis factor-α in human alveolar macrophages and peripheral blood monocytes: a cellular and molecular analysis. *Am. J. Respir. Cell. Mol. Biol.* **1,** 57.

VALONE, S.E., RICH, E.A., WALLIS, R.S. & ELLNER, J.J. (1988) Expression of tumor necrosis factor in vitro by human mononuclear phagocytes stimulated with whole mycobacterium bovis BCG and mycobacterial antigens. *Infect. Immun.* **56,** 3313.

WAAGE, A. & BAKKE, O. (1988) Glucocorticoids suppress the production of tumour necrosis factor by lipopolysaccharide-stimulated human monocytes. *Immunology*, **63,** 299.

WILLIAMS, G.T. & WILLIAMS, W.J. (1983) Granulomatous inflammation. a review. *J. clin. Pathol.* **36,** 723.

WRIGHT, S.C., JEWETT, A., MITSUYASU, R. & BONAVIDA, B. (1988) Spontaneous cytotoxicity and tumor necrosis factor production by peripheral blood monocytes from AIDS patients. *J. Immunol.* **141,** 99.